Comparative Genomics to Unravel the Role of Human Skin Microbiome Diversity and Immunogenetic Variation Associated with Differential Susceptibility to Rickettsial Pathogens

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1. Abstract

Background: Scrub typhus, caused by *Orientia tsutsugamushi*, is characterized by extensive strain variation, genomic diversity and variable clinical outcomes. The complex interplay among pathogen functional diversity, host HLA polymorphisms, and skin microbiome composition in relation to differential susceptibility to scrub typhus remains poorly understood.

Methods: Clinical samples from scrub typhus patients and healthy controls in and around Pondicherry were analyzed. Genotyping of *O. tsutsugamushi*, PCR-based HLA typing, and 16S rRNA amplicon sequencing for skin microbiome profiling were performed. Comparative genomic analysis, including phylogenetic and functional characterization, was conducted on 149 complete rickettsial genomes, including 17 *O. tsutsugamushi* genomes, along with 452 human skin-associated bacterial genomes. In addition, in silico antimicrobial peptide (AMP)-HLA binding affinity predictions were carried out to assess allele-specific immune interactions.

Results: PCR amplification and sequencing of the 56 kDa gene identified two distinct genotypes of *O. tsutsugamushi*, indicating genetic diversity among circulating strains in Puducherry region. Sequencing of the exon 2-3 region of HLA-A gene identified a non-synonymous Gly→Trp mutation in exon 3, associated with HLA-A*02 polymorphisms, observed predominantly in healthy individuals, suggesting a potential association with protective host immune response. Alleles such as HLA-A*24 and HLA-DRB1*15:01 were associated with disease susceptibility, while HLA-DRB1*17 was more frequent in healthy individuals. The skin microbiota profiling revealed no significant differences in overall diversity between scrub typhus patients and healthy individuals. However, specific taxa such as *Turicibacter*, *Neisseria*, *Fusobacterium*, and *Ezakiella* were enriched in healthy group, whereas *Rheinheimera* was associated with the scrub typhus group. Notably, several bacterial taxa showed significant associations with HLA-A*24, suggesting a potential role of host immunogenetic variation in shaping microbiome composition.

Comparative genomic analyses of Rickettsial species revealed phylogenetic discordance among few strains of *Rickettsia conorii* and *Rickettsia philipii*, and identified antimicrobial resistance gene (*alr*) and CAZyme families (GT51, GT30) as discriminatory markers absent in *O. tsutsugamushi*. Comparative genomic analysis of *O. tsutsugamushi* revealed higher inter-strain variation in virulence and key functional genes, which may contribute to strain-specific difference in host susceptibility. Functional analysis of human skin-associated bacteria showed

A. baumannii as a key reservoir of virulence genes, enriched in pilus and Type VI secretion system (T6SS) components, which are present in most pathogenic bacterial species. Additionally, strain-level variation in antimicrobial peptide abundance was observed in *O. tsutsugamushi* and certain microbial taxa associated with healthy and scrub typhus group, along with differential HLA-class I binding affinities, indicating allele-specific modulation of host immune responses.

Conclusion: These findings underscore a complex relationship among host immunogenetic variation (HLA diversity), functional diversity of *O. tsutsugamushi*, and host skin microbiome composition and diversity in shaping immune response and differential susceptibility to scrub typhus, provides important insights into disease pathogenesis and identify potential biomarkers, as well as potential diagnostic and therapeutic targets for improved management of scrub typhus.

Keywords: Scrub typhus, HLA, Skin Microbiota, Disease Susceptibility and Molecular Markers

2. Introduction

Ticks and mites are important arthropod vectors of infectious agents, transmitting pathogens to vertebrate host through prolonged attachment and sustained feeding on the host skin. During this process, pathogens are co-inoculated with vector saliva, making the skin the primary site of host-pathogen interaction. Increasing evidence highlights that the skin is not merely a physical barrier but an active immunological organ that initiates early innate immune responses, influencing pathogen establishment, replication, and persistence prior to systemic dissemination (Wikel, 2013; Boulanger and Wikel, 2021). Such early cutaneous events have been well documented in several vector-borne diseases, including Lyme borreliosis, spotted fever rickettsiosis, Jingmen tick virus infection, and scrub typhus, where the initial stages of infection are governed by complex interactions at the skin interface (Bernard et al., 2020; Heinze et al., 2014; Jia et al., 2019; Jia et al., 2020a). However, compared to tick-borne infections, mite-borne diseases such as scrub typhus, caused by *O. tsutsugamushi*, remain relatively underexplored, particularly in the context of early skin immune responses and host-microbiome interactions. The skin microbiome constitutes a dynamic and diverse community that plays a crucial role in maintaining immune homeostasis and modulating host defense mechanisms (Li et al., 2025). Its composition is influenced by both host genetic factors and environmental exposures (Byrd et al., 2018).

Emerging studies indicate that arthropod bites can alter the local skin microbiota, potentially impacting pathogen colonization and disease progression (Bernard et al., 2020, Boulanger and Wikel, 2021). The interaction between the microbiome and the host immune system is bidirectional, wherein microbial communities shape immune responses, while host immunity regulates microbial composition (Belkaid et al., 2014). Among host immunogenetic factors, human leukocyte antigen (HLA) genes are highly polymorphic and central to antigen presentation and adaptive immune responses (Trowsdale and Knight, 2013). Variations in HLA alleles have been strongly associated with susceptibility or resistance to a wide range of infectious diseases, including intracellular bacterial infections (Blackwell et al., 2009). In addition, functional diversity in *O. tsutsugamushi* strains can drive differential disease susceptibility by modulating antigenic variation and host immune recognition thereby contributing to differential susceptibility to scrub typhus disease. In this context, understanding the interplay between skin microbiome dynamics, inter and intra species functional diversity of rickettsial pathogens and host immunogenetic variation is critical for elucidating mechanisms underlying differential disease susceptibility in scrub typhus. Therefore, the present study aims to investigate the combined influence of skin microbiota diversity, Pathogen functional diversity and HLA diversity in determining host susceptibility to scrub typhus, with a particular focus on early host-pathogen interactions at the skin interface.

Research Gap/Rationale

The earliest host-pathogen interactions at the skin site of mite feeding, where the pathogen is inoculated with saliva, are key determinants of scrub typhus pathogenesis; however, their investigation in patients is limited by practical and ethical constraints. While early host-pathogen interactions have been well characterized in tick-borne diseases, they remain comparatively underexplored in mite-borne infections such as scrub typhus. Consequently, most studies focus on localized bite-site responses, leaving the broader skin microbiome diversity, Pathogens functional diversity, and the contribution of host HLA variation to differential disease susceptibility poorly understood .Therefore, studying the relationship between the overall skin microbiome and host immunogenetic variation is important to better understand differences in disease susceptibility and progression.

3. Aims and Objectives

Aim: To study the association of immunogenetic variation and skin microbiome diversity with differential susceptibility to rickettsial pathogen.

Objective 1: To study the immunogenetic variation in scrub typhus patients and healthy individuals.

Objective 2: To study the skin microbiome diversity and correlate the association of microbiome diversity and immunogenetic variation, with differential susceptibility to scrub typhus.

Objective 3: To reveal the comparative genomic analysis of skin bacteria and rickettsial pathogens with respect to virulence factor, antimicrobial peptides and antimicrobial resistant genes.

4. Hypothesis/Research Questions

4.1 Research Question: Is there an association between host skin microbiome compositions, HLA variation that can influence differential immune response in scrub typhus.

4.2 Research hypothesis: "Skin microbiome diversity and host Immunogenetic variation linked with differential immune response to rickettsial Diseases"

4.3 Null hypothesis: "No association between Skin microbiome diversity and host Immunogenetic variation with differential immune response to rickettsial diseases"

5. Review of Literature

5.1. Host skin microbiome and rickettsial disease

The skin microbiome plays a crucial role in vector-borne infections by influencing local inflammatory responses and potentially affecting arthropod feeding behavior and pathogen transmission at the bite site. Dysbiosis, or alterations in skin microbial composition, has been linked to increased susceptibility to skin infections and systemic immune-related conditions (Grice et al., 2008). Studies on tick bites and rickettsial infections have shown significant changes in the skin microbiome at the site of attachment (Boulanger et al., 2023). A meta-transcriptomic analysis of tick-bitten mice revealed a decrease in *Chlamydia* and an increase in genera such as *Streptococcus*, *Escherichia*, *Staphylococcus*, *Pseudomonas*, and *Acinetobacter*, which are associated with both tick microbiota and opportunistic infections. These shifts were

accompanied by functional changes, including enhanced genetic processing and signaling pathways, indicating an active microbial response to tick feeding. Additionally, *R. raoultii* infection was associated with enrichment of *Actinobacteria*-related metabolism and immune pathways such as focal adhesion and phagosome formation, highlighting complex interactions between the pathogen, microbiome, and host immunity (Du et al., 2023). Human studies further support these findings, showing overlap between tick and skin microbiota, including *Pseudomonas*, *Acinetobacter*, *Corynebacterium*, and *Propionibacterium*, suggesting microbial exchange during feeding. Co-infections with multiple pathogens were also common, reinforcing the role of skin as a reservoir for pathogen colonization. The detection of emerging pathogens such as *R. cervicalis* emphasizes the dynamic nature of the post-bite skin environment (Zhang et al., 2024). Unlike ticks, chiggers do not feed on blood but consume liquefied host tissue through a stylostome formed during feeding. Their saliva and enzymatic activity induce localized inflammation (trombiculiasis) (Chen and Roe, 2022; Shatrov and kudryashova, 2006). Although direct evidence is lacking, the close interaction of chiggers with skin tissue and their deposition of pathogen along with microbiota and immunomodulatory factors suggest a potential to alter the skin microbiome, similar to ticks. This highlights the need to investigate mite-associated microbiomes in scrub typhus to better understand vector-host-microbiome interactions and identify potential microbiome-based intervention strategies.

5.2. Role of MHC in scrub typhus Disease Pathogenesis

The *O. tsutsugamushi* employs sophisticated mechanisms to evade host immunity by targeting the major histocompatibility complex (MHC) pathway (Rodino et al., 2019). In non-professional antigen-presenting cells (APCs) such as endothelial and epithelial cells, the effector protein Ank5 binds to NLRC5, the master regulator of MHC class I genes, preventing its nuclear translocation. Ank5 also recruits the SCF ubiquitin ligase complex, promoting proteasomal degradation of NLRC5 and suppressing MHC-I transcription. This leads to reduced MHC-I surface expression and impaired antigen presentation to $CD8^+$ T cells, even under IFN-γ stimulation (Rodino et al., 2019; Adcox et al., 2024).This immunomodulation is strain- and cell type-dependent. Strains expressing ank5 show strong suppression of NLRC5 and MHC-I, whereas ank5-deficient strains exhibit weaker, Ank-independent effects (Adcox et al., 2024). In monocytes, a biphasic response is observed: early infection suppresses NLRC5 and MHC-I,

limiting $CD8^+$ T-cell activity, while later stages partially restore MHC-I expression through CIITA-mediated, NLRC5-independent pathways, enabling immune recognition (Rodino et al., 2019).

Ank5's dual structure-ankyrin repeats for NLRC5 binding and an F-box domain for ubiquitination is central to this process. By blocking NLRC5 nuclear import and promoting its degradation, *O. tsutsugamushi* reduces MHC-I expression and antigen presentation, facilitating persistence in non-professional APCs and contributing to disease severity. Partial recovery in monocytes is associated with improved pathogen clearance (Rodino et al., 2019; Adcox et al., 2024). In contrast, the pathogen upregulates MHC class II in professional APCs, indicating preferential survival in HLA-DR-expressing cells (Paris et al., 2012). This dual strategy-MHC-I suppression to evade $CD8^+$ T cells and MHC-II induction to engage $CD4^+$ T cells-reflects a balanced immune evasion mechanism.

5.3. Interconnection between skin microbiome and MHCs in immune activation

The skin microbiome plays a fundamental role in shaping host immunity through dynamic interactions with the host immune system. Commensal and pathogenic microbes influence immune responses partly via the production of antimicrobial peptides (AMPs), such as defensins and cathelicidins, which contribute to immune signaling and cellular recruitment (Zasloff, 2002). These peptides can attract antigen-presenting cells (APCs), including immature dendritic cells, B lymphocytes, and macrophages, and promote dendritic cell maturation through pathways involving Toll-like receptors and MyD88 signaling (Diamond et al., 2009; George et al., 2017). In addition, microbiome-derived signals play a key role in shaping T cell responses, often promoting immune profiles necessary for effective pathogen clearance. Central to this host-microbiome interaction is antigen presentation mediated by major histocompatibility complex (MHC) molecules, known as human leukocyte antigen (HLA) in humans. HLA molecules regulate the presentation of microbial and self-peptides to T cells, thereby influencing immune recognition, tolerance, and activation. The extensive polymorphism of HLA genes has been strongly associated with susceptibility or resistance to a wide range of infectious diseases, particularly intracellular infections (Blackwell et al., 2009; Trowsdale and Knight, 2013). Importantly, emerging evidence indicates that HLA variation can shape the composition and diversity of the human microbiome, including the skin microbiome, by modulating host immune

selection pressures on microbial communities (Bonder et al., 2016). Thus, the bidirectional interaction between the skin microbiome and HLA-mediated immune responses plays a critical role in maintaining immune homeostasis and determining disease susceptibility.

Connecting this to rickettsial infections, the skin microbiome's AMP-mediated recruitment and activation of APCs support the host defense by promoting effective MHC class II-mediated antigen presentation and consequential CD4+ T cell activation, vital for combating intracellular pathogens like *rickettsia*. By strengthening innate immunity and facilitating adaptive immune responses, AMPs help maintain skin barrier integrity and reinforce pathogen recognition and clearance. Disruptions in skin microbiota or AMP production may impair these immune mechanisms, weakening MHC expression and antigen presentation, thus increasing susceptibility to rickettsial infections particularly in *O. tsutsugamushi* infection. Therefore, the skin microbiome, through AMP-driven immunomodulation, forms a critical axis that enhances MHC-dependent immune defense against rickettsial and other intracellular pathogens, underscoring the importance of host-microbe interactions in immune protection.

6. Methodology

6.1 Sample size

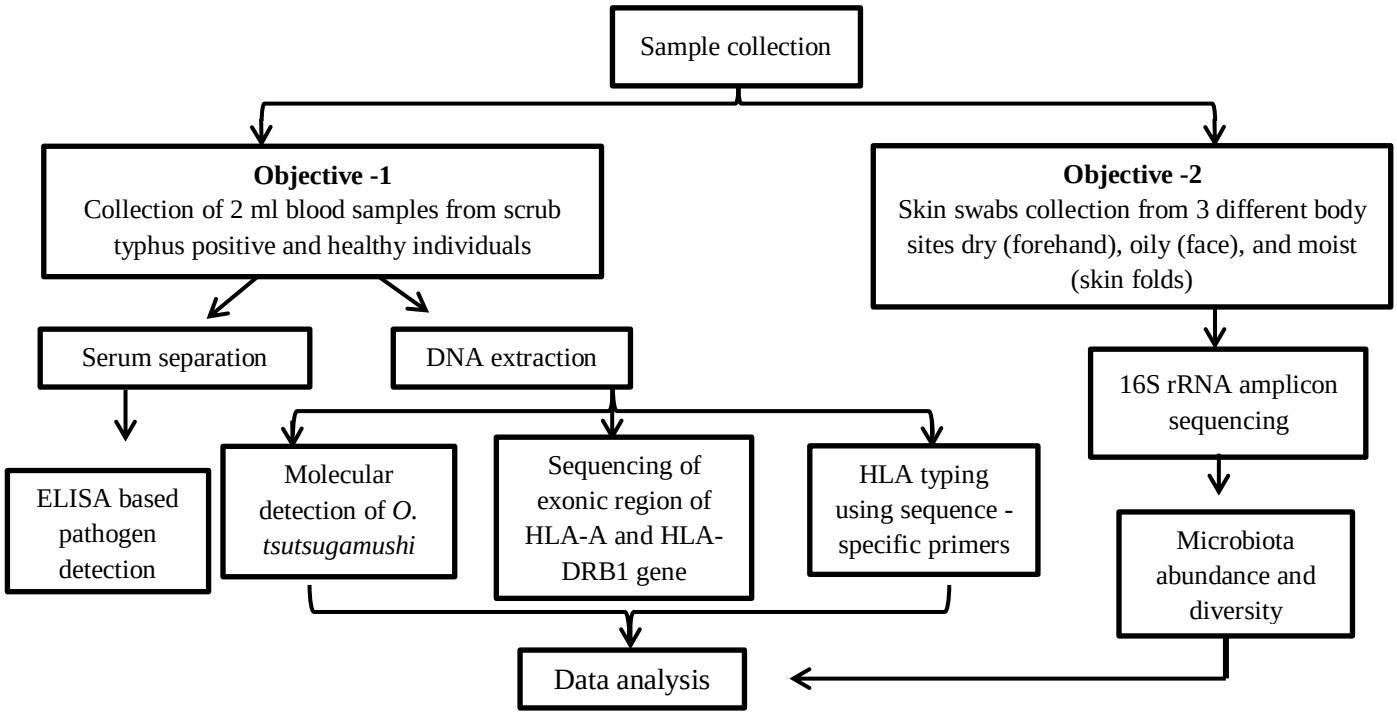
For Objective 1, an exploratory pilot study was conducted with 50 individuals in the disease group and 50 in the healthy group. For Objective 2, a pilot study was carried out with 15 individuals in the disease group and 15 in the healthy group.

6.2 Inclusion/Exclusion Criteria

6.2.1 Inclusion criteria: Patients above 12 years those who had been confirmed with scrub typhus infection will be included in this study.

6.2.2 Exclusion criteria: All the patients less than 12 years old and patients above 12 years with infectious diseases (other than scrub typhus) and systemic diseases will be excluded from the study.

6.2.3 Methodology flowchart for objective 1&2


Sample collection
Objective -1
Collection of 2 ml blood samples from scrub typhus positive and healthy individuals
Objective -2
Skin swabs collection from 3 different body sites dry (forehand), oily (face), and moist (skin folds)
Serum separation
DNA extraction
16S rRNA amplicon sequencing
ELISA based pathogen detection
Molecular detection of O. tsutsugamushi
Sequencing of exonic region of HLA-A and HLA-DRB1 gene
HLA typing using sequence - specific primers
Microbiota abundance and diversity
Data analysis

6.2.4 Methodology for objective 3

Complete genome sequences of rickettsial species and human skin-associated bacterial species were retrieved from public databases. Phylogenetic analysis of rickettsial species and *O. tsutsugamushi* was performed using 16S rRNA gene sequences and whole-genome sequences to determine evolutionary relationships. Functional profiling included antimicrobial resistance gene (ARG) screening and virulence factor identification. A comparative genomic analysis of *O. tsutsugamushi* was conducted to identify key genomic features associated with pathogenicity. For skin bacterial species, functional annotation was performed to identify antimicrobial resistance genes, virulence factors, and metabolic pathways using RAST subsystem categorization. Antimicrobial peptide (AMP) prediction was carried out for *O. tsutsugamushi* and selected skin bacteria associated with health and disease. Predicted peptides were further evaluated for HLA binding affinity and interferon-gamma (IFN-γ) inducing potential using in silico tools.

7. Results and discussion

7.1 Results for Objective 1: To study the association of Immunogenetic variation and skin microbiome diversity with differential susceptibility to Rickettsial pathogen.

7.1.1 Serological and Molecular Detection of *O. tsutsugamushi*

Out of 50 IgM card-positive samples, confirmation using ELISA and nested PCR targeting the 56 kDa gene of *O. tsutsugamushi* identified 38 and 11 positive cases, respectively, indicating differences in sensitivity between serological and molecular methods. Similar discrepancies have been reported in studies from Puducherry, likely reflecting variation in assay sensitivity and stage of infection. The 56 kDa gene amplicons were sequenced, and analysis of 11 samples revealed two distinct sequence patterns, indicating genetic variability. Phylogenetic analysis confirmed the presence of two genotypes circulating in and around Puducherry, consistent with previous reports (Anitharaj et al., 2020).

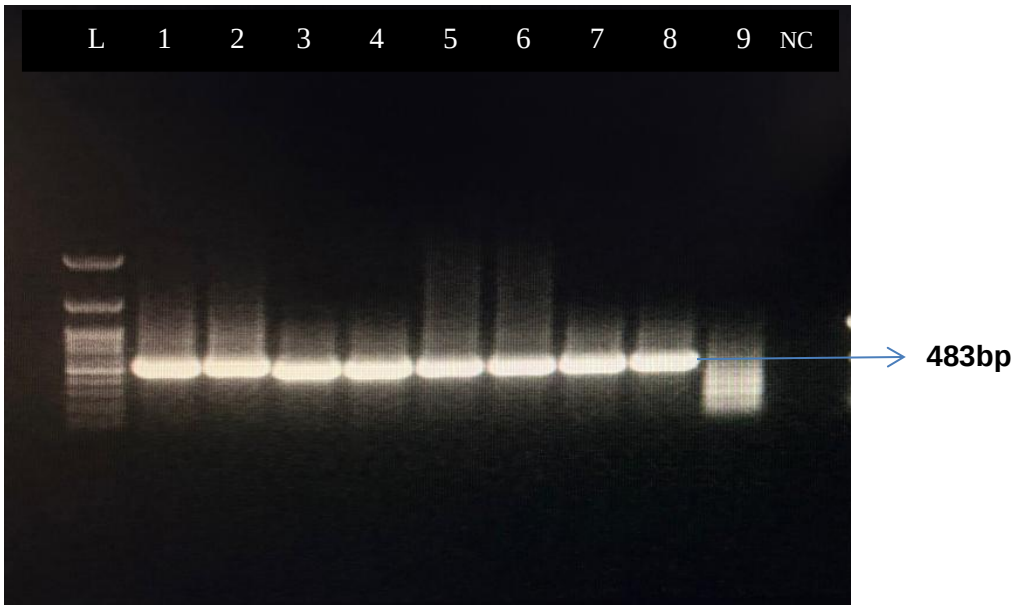


Figure 1: Amplification of 56 kDa gene using Nested PCR
L- 100 bp ladder, 1-9 – samples, NC- negative control

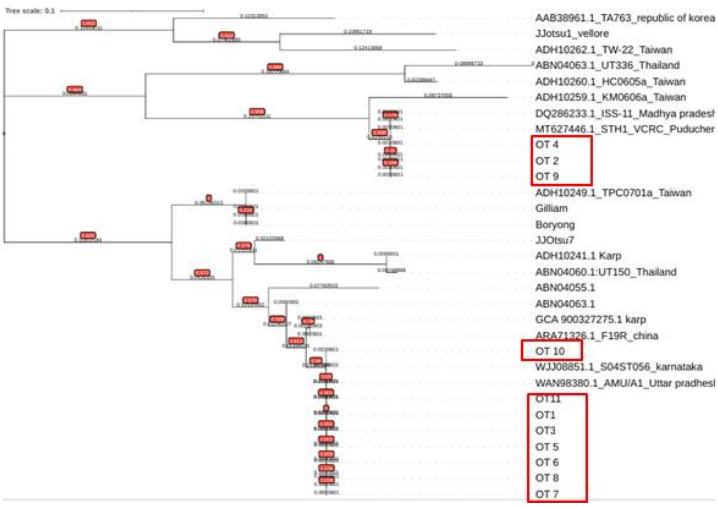


Figure 2: Phylogenetic tree construction using *the 56 kDa* gene of *O. tsutsugamushi identified* from the patient samples

Among these, three strains clustered with Puducherry isolates, while others grouped with strains from Karnataka and Uttar Pradesh, suggesting regional dissemination and shared evolutionary lineages.

7.1.2 Sequencing of exon 2 and 3 region of HLA-A gene

A total of 35 samples (20 diseases, 15 healthy) were analyzed. Exon 2 and exon 3 were amplified using specific primers-forward (AAACGGCCTCTGTGGGGAGAAGCAA) and reverse (GTGGCCCCTGGTACCCGT)-and sequenced. Multiple sequence alignment identified two point mutations at positions 205 (exon 2) and 597 (exon 3), both involving a guanine-to-thymine substitution, predominantly observed in healthy individuals. Translation showed that the exon 3 mutation at position 597 resulted in a non-synonymous amino acid change (glycine to tryptophan), while the exon 2 mutation did not alter the amino acid sequence. Comparative analysis indicated similarity with HLA-A*02 alleles. Since exons 2 and 3 encode the peptide-binding groove of HLA class I molecules, such variations can affect antigen presentation (Neefjes et al., 2011). The presence of the exon 3 mutation in healthy individuals suggests a

potential protective role, consistent with studies linking HLA-A*02 polymorphisms to enhanced immune responses and resistance to infections (Blackwell et al., 2009).

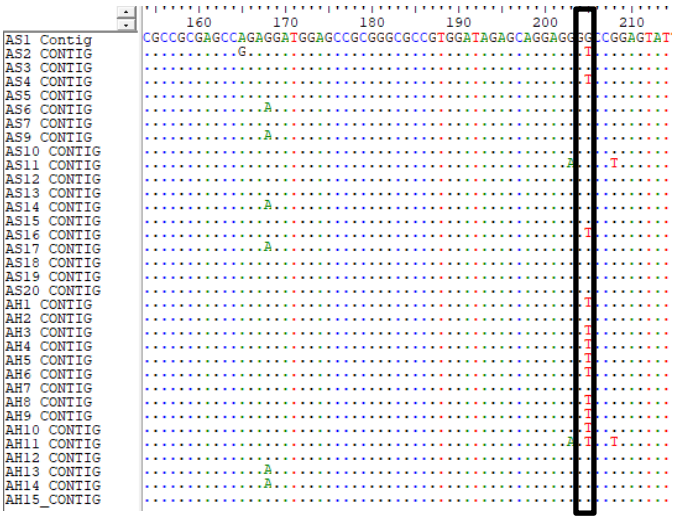


Figure 3a: Multiple sequence alignment of exon-2 region of HLA-A gene indicating the presence of G205T mutation

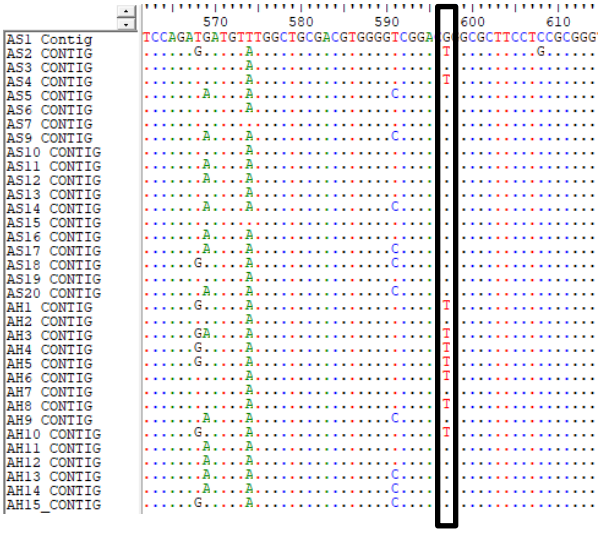


Figure 3b: Multiple sequence alignment of exon-3 region of HLA-A gene indicating the presence of G597T mutation

7.1.3 Sequencing of exon 3 region of HLA-DRB1 gene

A total of 35 samples (20 diseases, 15 healthy) were analyzed for HLA-DRB1 variation. The exon 3 region was amplified using specific primers (Forward: TCCTGACTCATTCCCTCTACCT; Reverse: GAAGTCAGAAAGCTGCTC) and sequenced. Multiple sequence alignment revealed high polymorphism across all samples, consistent with the known variability of HLA-DRB1 alleles. However, no mutations were uniquely associated with either the disease or healthy groups. HLA-DRB1 is highly diverse and plays a key role in immune response variation. The absence of group-specific mutations suggests that exon 3 variations may not directly influence disease susceptibility or protection in this population, consistent with previous findings (Blackwell et al., 2009)

Figure 4: Multiple sequence alignment of exon-2 region of HLA-DRB1 gene

7.1.4 HLA typing using Sequence-specific primers

HLA typing was carried out for the alleles listed in the table below, including their respective annealing temperatures and expected product sizes (in base pairs)

Table 1: The primers used for HLA typing used in this study was listed below

Sl. no	HLA allele	Primer sequence	Ta (°C)	Product size
1	HLA-B*40	FP-CGAGAGAGCCTGCGGAAC	61	1331
		RP-GCCCACTTCTGGAAGGTTCT		
2	HLA-A*24	FP-CCTCCAGGTAGGCTCTCTG	53	559
		RP-GGCCGGAGTATTGGGACGA		
3	HLA-A*10	FP- ATGTAATCCTTGCCGTCGTAA	57	217
		RP-GGAGTATTGGGACCGGAAC		
4	HLA_DRB1*1501	FP-CGAGAGAGCCTGCGGAAC	62	197
		RP-CCGCGCCTGCTCCAGGAT		
5	HLA-DRB1*17	FP-GACGGAGCGGGTGCGGTA	51	217
		RP-CTGCACTGTGAAGCTCTCCA		

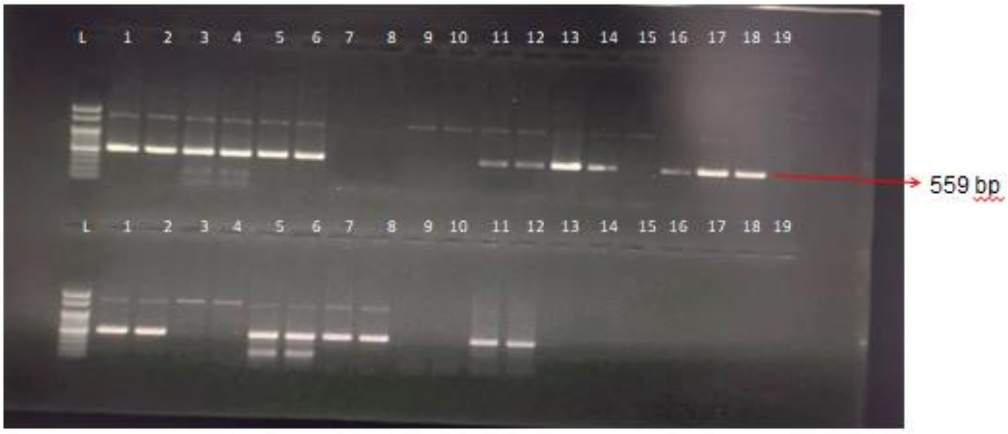


Figure 5: PCR amplification of HLA-A*24 alleles
L- 100 bp ladder, 1-18- samples, 19- Negative control

The HLA-A*24 allele is significantly associated with scrub typhus, being more frequent in patients (74%) than in healthy individuals (40%). The low p-value (0.0006) confirms statistical significance, and the odds ratio (4.269) suggests that carriers have about 4.3 times higher risk, indicating a potential susceptibility factor.

Table 2: Association of HLA alleles with Scrub typhus and healthy individuals

Alleles	Status	Positive	Negative	P-value	Odds ratio	Results
HLA-A*24	Scrub typhus	37/50	13/50	0.0006	4.269	Associated with Scrub typhus
	Healthy	20/50	30/50			
HLA-B*40	Scrub typhus	27/50	23/50	0.5529	0.7826	No association
	Healthy	30/50	20/50			
HLA-A*10	Scrub typhus	34/50	16/50	0.27	0.5994	No association
	Healthy	39/50	11/50			
HLA-DRB1*1501	Scrub typhus	48/50	2/50	0.008	6.769	Associated with Scrub typhus
	Healthy	39/50	11/50			
HLA-DRB1*17	Scrub typhus	27/50	23/50	0.02	0.3707	Associated with Healthy group
	Healthy	38/50	12/50			

7.2 Results for Objective 2: To study the skin microbiome diversity and correlate the association of microbiome diversity and immunogenetic variation, with differential susceptibility to scrub typhus

7.1.1 Relative abundance of Skin microbiota diversity between healthy and scrub typhus group

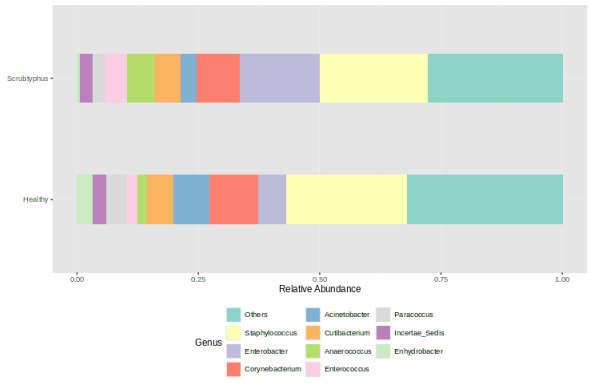


Figure 6: Relative abundance of skin microbiota at genus level between the disease and healthy

At the genus level, scrub typhus samples showed increased relative abundance of *Enterobacter*, *Enterococcus*, and *Anaerococcus*, indicating enrichment of opportunistic taxa in scrub typhus. In contrast, healthy samples were enriched with *Staphylococcus*, *Corynebacterium*, *Acinetobacter*, *Enhydrobacter*, and *Paracoccus*, which are typical commensal microbiota, highlighting distinct microbial patterns between the two groups.

7.1.2 Alpha diversity of skin microbiota at genus level

P-value: 0.85

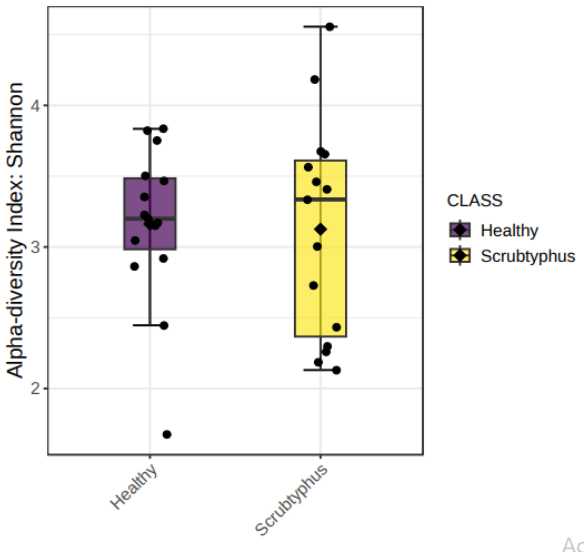


Figure 6a: Alpha diversity at genus level between healthy and scrub typhus group

P-value: 0.69

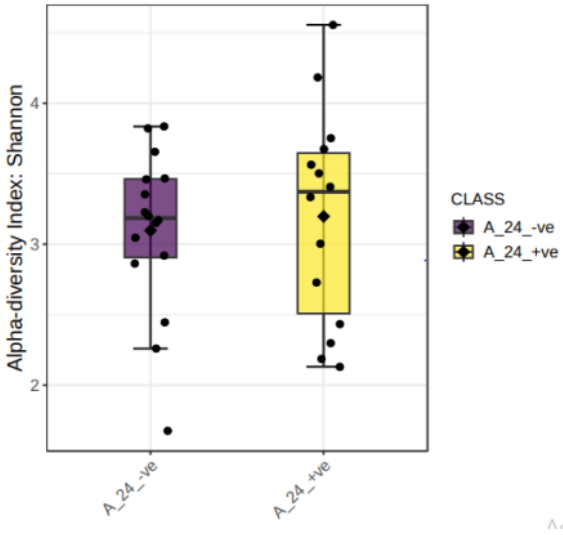


Figure 6b: Alpha diversity at genus level between HLA-A*24 positive and negative

No significant variation was observed in microbial diversity between disease and healthy groups or between HLA-A*24 positive and negative groups, indicating that overall microbiota diversity is stable between conditions despite differences in taxonomic composition.

7.1.3 Differential Microbial Signatures Associated with Disease Status and HLA-A*24

LEfSe analysis comparing healthy and disease groups identified five significantly discriminative microbial taxa (adjusted p-value < 0.05; log LDA score ≥ 2.0). Among these, *Neisseria*, *Turicibacter*, *Fusobacterium*, and *Ezakiella* were enriched in the healthy group, whereas *Rheinheimera* was associated with the disease group, indicating distinct microbial signatures linked to disease status.

To further assess the influence of HLA and differential abundance analysis using MaAsLin2 was performed at the genus level to examine associations with HLA-A*24 status, independent of disease and health status. A total of 206 microbial features were analyzed using a negative binomial model with an FDR-adjusted significance threshold of 0.05. Among these, 17 genera remained significantly associated with HLA-A*24 status after FDR correction ($q < 0.05$), indicating robust association's independent of disease status. Although one feature was significant at the raw p-value level, it did not retain significance after correction and was excluded. These results suggest that, beyond disease-associated microbial variation, host HLA-A*24 status plays a significant role in shaping microbiome composition and diversity, independent of scrub typhus infection

3. Results for Objective 3: To reveal the comparative genomic analysis of skin bacteria and rickettsial pathogens with respect to virulence factor, antimicrobial peptides and antimicrobial resistant genes.

7.3.1 Comparative genomic analysis of Rickettsial species

7.3.1.1 Retrieval and annotation of Rickettsial genomes

The Rickettsial genomes were retrieved from the National Center for Biotechnology Information database. As of December 2024, a total of 349 rickettsial genomes were available, of which 149 were complete genomes representing 30 distinct *Rickettsia* species were taken for the analysis.

All selected genomes were individually annotated and subsequently used for downstream functional analysis.

7.3.1.2 Phylogenetic Analysis of Rickettsial Genomes

Phylogenetic analysis of 149 complete Rickettsial genomes revealed consistent clustering patterns between 16S rRNA and whole-genome based phylogeny. The 16S rRNA-based phylogeny identified seven distinct genus-specific clusters, with clear species-level grouping; however, notable variations were observed among certain strains. In particular, the 16S rRNA tree indicated potential misclassification which may support reclassification of specific strains, including *R. conorii*, *R. philipii*, and several Rickettsial endosymbionts. Whole-genome-based phylogenetic reconstruction also resolved seven major clusters, consistent with genus-level delineation. Additionally, strain-specific clustering was evident, aligning with the relationships inferred from 16S rRNA analysis. Overall, genome-scale phylogeny provided higher resolution and supported the observed taxonomic inconsistencies highlighted by 16S rRNA-based analysis.

Figure 7a: 16S rRNA based phylogeny of 149 Rickettsial species

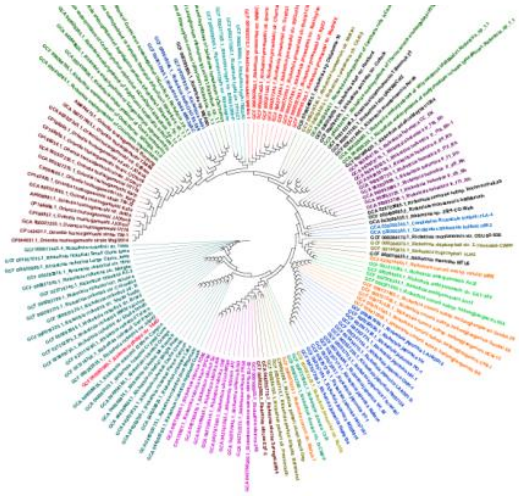
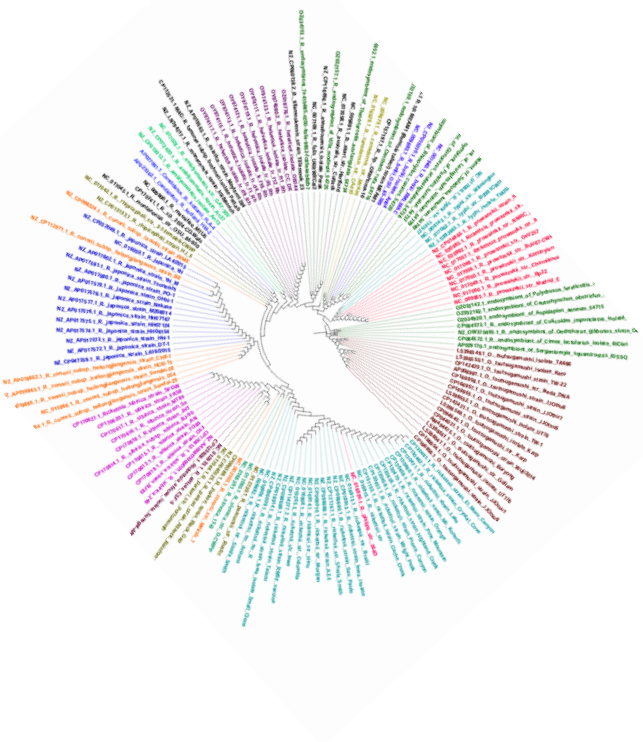


Figure 7b: whole genome-based phylogeny of 149 Rickettsial species



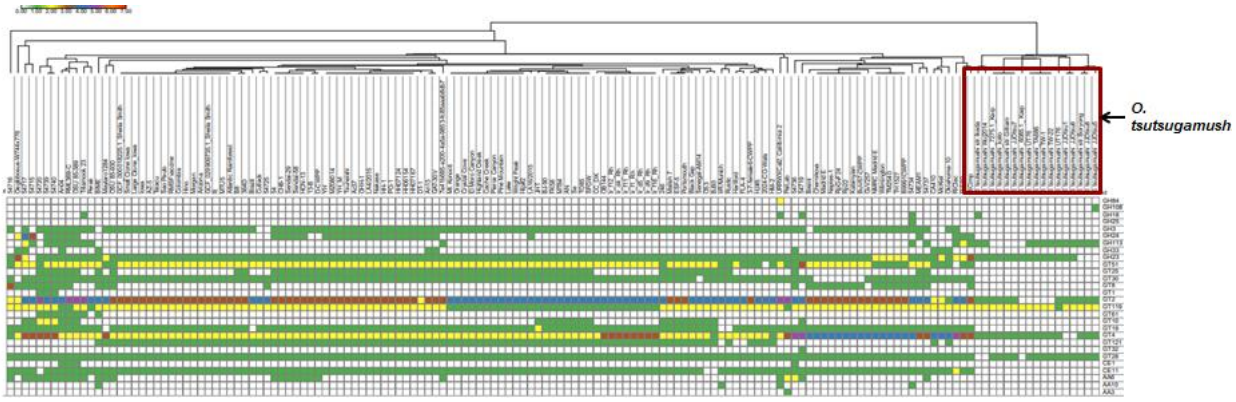
7.3.1.3 Functional analysis of Rickettsial genomes

A total of 28 RAST subsystems revealed clear functional differences between *Orientia* with other *Rickettsia* pathogens, particularly in gene copy number variation. Other Rickettsial species showed enrichment in fatty acid, lipid, and isoprenoid metabolism, indicating greater metabolic autonomy, whereas these pathways were reduced in *Orientia*, consistent with its obligate intracellular lifestyle (Nakayama et al., 2008). In contrast, *Orientia* exhibited increased membrane transport functions, suggesting reliance on host-derived nutrients (Nakayama et al., 2008). Phage-related elements in both genera indicate horizontal gene transfer contributing to genome diversification.

ARG profiling across 149 rickettsial genomes identified 22 resistance genes with largely conserved distribution. The *alr* gene was consistently present in other rickettsial species but absent in *Orientia*, supporting its use as a genus-level marker, while variation in Class D β-lactamase suggests lineage-specific adaptation. The absence of these genes in *Orientia* aligns with its reduced and modified peptidoglycan-like system (Nakayama et al., 2008; Atwal et al., 2017), whereas *Rickettsia* retains a more conserved cell envelope pathway (Drancourt & Raoult, 1999).

A total of 28 CAZyme subfamilies were identified in 149 rickettsial genomes, with a markedly reduced presence of CAZymes in *Orientia*. The absence of GT30 and GT51 family enzymes indicates limited glycan biosynthesis and a minimal cell wall structure. In contrast, other rickettsial species retains GT51, supporting functional peptidoglycan synthesis. The consistent absence of GT30 and GT51 in *Orientia* highlights their potential as discriminatory genomic markers, reflecting genome reduction and host dependency.

Figure 8: Distribution of CAZymes across 149 Rickettsial genomes



7.3.1.4 Comparative genomic analysis of *Orientia tsutsugamushi* genomes

Integrated phylogenetic and functional analyses of 17 *O. tsutsugamushi* strains, including ARG profiling, CAZyme distribution, RAST, COG categories, TCA cycle enzymes, and virulence factors, revealed high intra-species variation. This diversity may reflect genome plasticity driven by gene loss, duplication, and horizontal gene transfer (Nakayama et al., 2008). Variations in metabolic pathways and virulence-associated genes suggest strain-specific adaptations influencing host-pathogen interactions and immune evasion (Kelly et al., 2009). This heterogeneity likely contributes to differences in pathogenic potential, leading to variable clinical outcomes and differential host susceptibility to scrub typhus infection.

7.3.2 Comparative genomic analysis of Skin bacterial genomes

7.3.2.1 Functional analysis of skin bacterial genomes

Functional genomic analysis of 452 human skin bacterial genomes revealed distinct patterns across ARGs, metabolic functions, and virulence profiles. ARG profiling identified *arnT* and *sdrM* as potential genus-specific markers, with *arnT* associated with *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and some *Acinetobacter baumannii* strains, while *sdrM* was specific to the *Staphylococcus* genus. Lineage-specific markers such as vanW in *P. aeruginosa* and vanT in *S. aureus*, along with their absence in beneficial bacteria, highlight their diagnostic value. These findings suggest that ARGs can serve as reliable molecular markers for genus- and lineage-level discrimination.

RAST subsystem analysis showed reduced secondary metabolism in *Cutibacterium acnes* and *Streptococcus anginosus*. Additionally, the absence of subsystem categories related to cell division and cell cycle regulation in several commensals, including *Corynebacterium, Micrococcus, Moraxella*, and *Roseomonas*, as well as in certain opportunistic pathogens such as *A. baumannii* and *P. aeruginosa*, may indicate a slow-growing or a metabolically constrained lifestyle. This functional reduction in some skin-associated bacterial genomes likely reflects adaptation to stable host-associated niches.

Virulence factors profiling reveals that *A. baumannii* acts as a core reservoir of pathogenic genes. Key virulence determinants such as pili related genes and Type VI secretion system (T6SS) components (TssA, TssC, TssG) were common among major pathogens (*A. baumannii*, *P. aeruginosa*, *K. pneumoniae*), while ompA and clpU/tssH served as shared species-level markers.

In contrast, these virulence genes were largely absent in commensals. Overall, the differential distribution of virulence factors clearly distinguishes pathogenic from commensal skin microbiota, supporting their use as functional and diagnostic markers.

7.3.3 Screening of AMP and HLA binding Properties with INF- γ a inducing properties

Protein sequences from *O. tsutsugamushi* and skin-associated bacteria associated with health and disease were analyzed for antimicrobial peptide (AMP) prediction. The predicted AMPs were further evaluated for HLA class I binding affinity and IFN-γ inducing potential. The analysis identified a subset of peptides with the capacity to bind HLA molecules and potentially elicit immune responses. These findings suggest that such peptides may play a role in host immune modulation and can be explored further for their immunogenic and therapeutic potential in future studies.

8. Summary and conclusion

This study highlights the complex interplay among the functional genomic diversity of rickettsial pathogens, host immunogenetic variations, and skin microbiome diversity in determining susceptibility to scrub typhus. Two distinct genotypes of *O. tsutsugamushi* identified in and around Pondicherry indicate substantial genetic variability that may influence disease outcomes. HLA polymorphism analysis revealed that HLA-A*24 and HLA-DRB1*15:01 are associated with disease susceptibility, whereas HLA-DRB1*17 is more frequent in healthy individuals. A non-synonymous Gly→Trp mutation in exon 3 of the HLA-A gene, corresponding to HLA-A*02 polymorphisms, was observed in most of healthy individuals, suggesting a potential protective role. While overall skin microbiome diversity did not differ significantly, specific genera were differentially enriched and associated with HLA status, indicating the influence of host immunogenetic variation on microbial composition. Comparative genomic analysis of rickettsial species underscored taxonomic inconsistency in the placement of certain strains of *R. philipii* and *R. conorii*. The presence of the *alr*, an antimicrobial resistance gene and CAZyme families GT51 and GT30 in other rickettsial species but not in *O. tsutsugamushi* highlights potential genus specific molecular markers. Functional analysis of 452 skin bacterial genomes revealed *Acinetobacter baumannii* as a key reservoir of virulence genes, with enrichment of pilus and T6SS components in pathogenic strains compared to commensals. Antimicrobial peptides

derived from *O. tsutsugamushi* and associated microbiota showed HLA-binding and INF- γ inducing potential, suggesting their role in host immune modulation and disease pathogenesis.

9. Limitation

- The microbiome analysis was limited by a relatively small sample size with study participants from single tertiary care hospital, which may affect the statistical power and generalizability of the findings.
- Several findings, particularly AMP prediction and comparative genomic insights, are based on in silico approaches and require experimental (wet lab) validation.

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11. Publication

1. **Jayachandiran S**, Suresh R**,** Dhamodharan R. Comparative and phylogenomic analysis of *Chlamydia pneumoniae* reveals unique carbohydrate-active enzyme family (GT5) among respiratory isolates. ***Infection, Genetics and Evolution***. 2025 Aug 21:105813. (**First-Author**)

2. **Jayachandiran S**, Suresh R**,** Ramasamy D. Bacteriome in Health & Diseases, 'Textbook of Bacterial Zoonoses" ***Book chapter, Springer Nature.*** (**First-Author**) - Accepted (In Press)

3. **Jayachandiran S**, Suresh R, Chakraborty M, Lakshmanan D**,** Dhamodharan R. Phylogenomics and Comparative Genomics of Colistin-Resistant Chryseobacterium indologenes Highlight Strain-Specific Diversity and Support the Taxonomic Revision of Strain AA5 as *C. metallocongregator* and PgBE117 and Ery-6B as Putative Novel Species. ***Microbiology.*** **(First-Author)-** Accepted for publication.

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7. Suresh R, Jayachandiran S, Balu P, Ramasamy D. Comparative and Phylogenomic Analysis of the *Eikenella* Genus Suggests Taxonomic Reclassification of *Eikenella corrodens* Strain KCOM 3110 as a Novel Species and Reveals Unique CE1 Carbohydrate-Active Enzyme Family. ***Molecular Genetics and Genomics*** 2026 Dec;301(1):20. (**Co-Author**)

12. Oral and Poster presentation

- Presented a poster on rickettsial resistome profiling at the International Conference on Emerging and Re-emerging Infectious Diseases (ICER-ID 2025).
- Oral presentation on comparative genomics of *Orientia tsutsugamushi* at International Conference on Pathology & Genomics for Precision Medicine (PAGE-25).
- Presented a poster on Phylogenomics and resistome profiling of *Corynebacterium diphtheriae* at the 6th MICROSUMMIT 2025 International Conference.

First author

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Research paper

Comparative and phylogenomic analysis of *Chlamydia pneumoniae* reveals unique carbohydrate active enzyme family (GT5) among respiratory isolates

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ABSTRACT

Chlamydia pneumoniae is an obligatory intracellular pathogen found in humans and animals. Understanding the genomic diversity is crucial for unravelling its pathogenic mechanisms and transmission dynamics. In this study, 14 complete genomes of *C. pneumoniae* strains were compared for functional diversity analysis. The koala isolate LPCoLN appears as a phylogenetically distinct, showing the fewest accessory genes and the highest incorporation of unique or absent genes among the strains analyzed. Functional annotation indicates that certain metabolic pathways between the LPCoLN and the human respiratory strain AR39 were the same, which is most likely due to phage-associated elements present in AR39. The presence of the GT5 CAZyme family is significantly associated with strains of respiratory origin, suggesting a potential role in respiratory adaptation and pathogenic strategies, including tissue colonization, immune evasion, and niche-specific persistence. The strong association between GT5 CAZymes and respiratory-origin strains highlights their potential as diagnostic markers and therapeutic targets.

1. Introduction

Chlamydia pneumoniae is an obligate intracellular bacterium responsible for a wide range of respiratory infections, such as pneumonia, bronchitis, and sinusitis, and has been linked to chronic conditions such as atherosclerosis (Balin et al., 2018). It primarily affects humans and is transmitted via respiratory droplets, making it a significant cause of community-acquired pneumonia worldwide. Recent studies show that *C. pneumoniae* accounts for about 1–2 % of community-acquired pneumonia (CAP) cases in children worldwide, with some regions reporting higher rates, for example, 16.2 % prevalence among children under 6 in Mexico (Vieyra et al., 2023). In India, limited data indicate a notable presence of *C. pneumoniae* in pediatric CAP cases, with 8.5 % positivity reported in hospitalized children from Thanjavur, Tamil Nadu (Ramamoorthy and Sundaram, 2018). Serological studies have shown that *C. pneumoniae* is widely prevalent in the general population, with many individuals having antibodies against this bacterium, indicating past exposure to this infection (Tuuminen et al., 2000; Ben-Yaakov et al., 2002). Some strains of *C. pneumoniae* have been isolated from sites other than the respiratory tract, such as arteries, eye conjunctiva and animal sources such as horse, dogs, koala, frogs, snakes and iguanas (Kuo et al., 1986; Liu et al., 1986; Berger et al., 1999; Wardrop et al., 1999; Bodetti et al., 2002). Furthermore, *C. pneumoniae* has been identified in respiratory samples, such as pharyngeal swabs and bronchial lavage, from individuals with pneumonia, bronchitis, and sinusitis (Kuo et al., 1995). Bodetti et al. (2002) reported six cases of disease/death in various animal species caused by *C. pneumoniae* infection, suggesting that mammals, amphibians, reptiles, and other animals could serve as the natural reservoir for this bacterium.

Once known as *Chlamydophila pneumoniae*, this pathogen is often

Abbreviations: 16S rRNA, 16S Ribosomal ribonucleic acid; ANI, Average Nucleotide Identity; ARG, Antimicrobial resistance genes; BPGA, Bacterial Pan Genome Analysis; CARD, Comprehensive Antibiotic Resistance Database; CAP, Community-acquired pneumonia; CAZymes, Carbohydrate active enzymes; CDSs, Coding sequences; COGs, cluster of orthologous groups; DDH, DNA-DNA Hybridisation; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; GC, Guanine-Cytosine; GT, Glycosyltransferase; GH, Glycoside Hydrolases; CE, Carbohydrate Esterases; KEGG, Kyoto Encyclopedia of Genes and Genomes; NCBI, National Centre for Biotechnology Information; PATRIC, Pathosystems Resource Integration Centre; pfkA, Phosphofructokinase 1; Pfp, Diphosphate-dependent phosphofructokinase; PGK, Phosphoglycerate kinase; PK, Pyruvate kinase; RAST, Rapid Annotations using Subsystems Technology; T3SS, type III secretion system.

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CHAPTER 50

Bacteriome in Health and Diseases

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Abstract

Zoonotic diseases are a major and increasing global health threat, affecting all sectors of human society, representing an estimated 61% of human pathogens and 75% of all emerging infectious diseases. The human microbiome is likely to play a central role in determining host susceptibility and immunity against these pathogens, which are altered and compromised due to various biotic and abiotic factors. Such changes, referred to as microbial dysbiosis, are an imbalance in the complex ecosystem of the human microbiota. When microbial dysbiosis occurs, colonisation resistance and immune homeostasis are interrupted, which can increase susceptibility to pathogen invasion and severity of disease outcomes. An individual with a compromised bacteriome may be at an increased risk of zoonotic infection due to the impairment of the immune response associated with the disrupted bacteriome. The field of microbiome research has emerged to include not only novel diagnostic methods and therapeutics for zoonotic infections, such as probiotics, faecal microbiota transplantation (FMT), and engineered bacteriophages (Phage therapy), to restore microbial balance and enhance host defence systems. Targeted strategies, including prebiotics and

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METADATA

Title:

Phylogenomics and Comparative Genomics of Colistin-Resistant Chryseobacterium indologenes Highlight Strain-Specific Diversity and Support the Taxonomic Revision of Strain AA5 as C. metallocongregator and PgBE117 and Ery-6B as Putative Novel Species

Abstract:

Chryseobacterium indologenes is an opportunistic pathogen with complex taxonomy due to genomic diversity and ecological variation. This study analyzed 24 genomes, including three environmental isolates (AA5, Ery-6B and PgBE117) from plant, root and soil, and 21 clinical isolates using phylogenetic, phylogenomic, and functional analyses. Phylogenetic and genomic data separated the environmental strains from the main C. indologenes cluster. AA5 showed close relation to C. metallocongregator, while Ery-6B and PgBE117 constitute novel taxa. Functional profiling revealed a conserved metabolic core and colistin resistance, but distinct CAZyme repertoires in environmental isolates, indicating adaptations to plant- and soil-derived polysaccharides. Genomic islands associated with mobile genetic elements, antimicrobial resistance, and virulence factors contribute significantly to genomic variability and enhance ecological fitness. These genomic and functional distinctions support taxonomic reassignment of C. indologenes AA5 as C. metallocongregator and classify C. indologenes Ery-6B and PgBE117 as new Chryseobacterium species. This clarifies the boundary between clinical and environmental lineages, enhancing the understanding of Chryseobacterium diversity and ecology

Funding Information

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Reviews

COMMENT1

Thank you for the detailed reply to my questions. T
ow be accepted after addressing one minor comm
e heatmap depicts the distribution of genomic islai
enes across all strains. It is very interesting how th
fied the genomic islands — that is, what exactly we
island-associated genes? Additionally, in its curren
e is not readable and nothing is understandable. I r
luding it.

Read Less

Completion of mandatory course work

SWAYAM ONLINE COURSE CERTIFICATION

This Certificate is awarded to

SUSANTHIKA J

for successfully completing the 2 credit course

Research and Publication Ethics (RPE)

with a consolidated score of 83% marks

in the proctored examination held on 01.12.2023

offered by ***Dr. Anirban Ghosh*** *of*

Netaji Subhas Open University, Kolkata

Roll No:TN01010006

Prof. Uma Kanjilal
National Coordinator
Indira Gandhi National Open University, New Delhi

Dr. Ashit Baran Aich
Registrar(Acting)
Netaji Subhas Open University, Kolkata

Issued On: 25-12-2023

To validate and check scores: https://swayam.gov.in/

Completed the SWAYAM course on Research and Publication Ethics with 83% marks.

SRI BALAJI VIDYAPEETH

DEEMED TO BE UNIVERSITY

Certificate

SBV Credit 4

This is to certify that

Dr./Mr./Ms. SUSANTHIKA J

UIN. 2205003006 has attended, actively participated and fulfilled the requirements as per University norms of the online workshop on

"RESEARCH METHODOLOGY, BIOSTATISTICS AND PREPARATION OF SCIENTIFIC MANUSCRIPT"

conducted by Sri Balaji Vidyapeeth, Puducherry

from 19.04.2023 to 25.05.2023.

Dr. G. Ezhumalai
Course Co-ordinator,
SBV

Dr. K.S. Reddy
Dean, Research
SBV

Prof. Nihar Ranjan Biswas
Vice Chancellor,
SBV

Completed mandatory coursework on Research Methodology, Biostatistics, and Preparation of Scientific Manuscripts offered by SBV

International conferences attended

Poster Presentation at the International Conference on Emerging and Re-emerging Infectious Diseases (ICER-ID 2025)

iCER-ID 2025
International Conference on Emerging and Re-emerging Infectious Diseases
(Jointly organized by Dr ALM PG IBMS, University of Madras & Infectious Diseases Society of India)
CERTIFICATE OF APPRECIATION
This is to certify that
Ms. SUSANTHIKA. J
has participated and presented a research poster entitled
Phylogenomic and resistome profiling of 149 rickettsial genomes
at the International Conference on Emerging and Re-emerging Infectious Diseases (iCER-ID 2025) held on 17th and 18th February 2025, at CSIR-SERC, Taramani, Chennai, India.
Dr.E.M.Shankar
President - IDSI
Chennai
Dr.S.Raju
Organizing Secretary - IDSI
Chennai
Dr.D.Prabu
HOD (I/c) Dept. of Microbiology
Dr. ALM PG IBMS, University of Madras
Dr.S.Elumalai
Registrar
University of Madras

Oral presentation at International Conference on Pathology & Genomics for Precision Medicine (PAGE-25)

VINAYAKA MISSION'S RESEARCH FOUNDATION
AVMC
AARUPADAI VEEDU MEDICAL COLLEGE
PAGE-25
FUTURE OF DIAGNOSTICS
International conference on
Pathology & Genomics for Precision Medicine
Certificate of Participation
This is to certify that
Ms. SUSANTHIKA. J
presented his/her research in the Oral/~~Poster~~ Presentation session at PAGE-25, organized by the Departments of Pathology and Medical Biotechnology, Aarupadai Veedu Medical College & Hospital, Puducherry, India, on 16th May 2025.
Dr. J. Jeyashambavi
Organizing Secretary
Dr. S. Ravikumar
Organizing Chairperson
Dr. R. Jawahar
Organizing Chairperson
Dr. T. Rajan
Vice Dean (Admin), AVMCH
Dr. Rakesh Sehgal
Dean, AVMCH

Poster presentation at 6th MICROSUMMIT 2025 International Conference

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Anusandhan National Research Foundation
6TH MICROSUMMIT 2025
CERTIFICATE OF PRESENTATION
10
This is to certify that
Ms. SUSANTHIKA J
has presented a paper/ poster at the 6th MICROSUMMIT-2025: International Conference on Cutting-Edge Strategies in Epidemiology and Diagnostics of Infectious Diseases (CESEDID-2025) held at Saveetha Dental College & Hospitals, Chennai, on the 1st and 2nd of August, 2025, organized by the Department of Microbiology in association with the Indian Association of Applied Microbiologists (IAAM), and co-sponsored by ANRF and CSIR, New Delhi.
Dr. S. Aravind Kumar
Dean, SDC
Dr. A. W. Santhosh Kumar
President, IAAM
Dr. Rajesh Kanna
Convenor - CESEDID-2025
Dr. A. S. Smiline Girija
Organizing Chair
Prof & Head - Microbiology